Residual antimicrobial effect of week organic acids on spoilage psychrotrophs at pig carcasses

Sorin Daniel DAN*[1], Marian MIHAIU[1], Oana REGET[1], Alexandra TĂBĂRAN[1]
[1]University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Department of Animal Production and Food Safety, 3-5 Mănăştur Street, Cluj-Napoca, Romania, sorindan@usamvcluj.ro

Abstract

The aim of this researchwas to assess the residual antimicrobial effect of 3% lactic and acetic acid solutions regarding the load and configuration of psychrotrophs at porcine carcasses. During October 2016 and December 2016, 18 pork meat samples were collected from a comercial slaughterhouse in Transylvania. Collected samples were sprayed with 3% acetic acid and lactic acid solutions by spraying on the surface of meat samples. Each sample was divided into three sub-samples, from which two were treated with 3% organic acids solutions and one was the control sample. Experimental design were carried out over a 14-day period with microbial analyses at day 0, 1, 5, 9, 14. After spraying with organic acid solutions, the samples were kept at 2-4°C for 24 hours, and the following microbiological determinations were carried out: total load of psychrotrophic germs and isolation of microorganisms from the genera Pseudomonas, Aeromonas, Yersinia and Enterobacteriaceae family. The most sensitive psychrophic bacteria regarding the decontamination effect of lactic and acetic acid were Aeromonas spp. and Yersinia spp., both species being completely inhibited after 24 hours since application. For all microbiological criteria analyzed, lactic and acetic acid shown an obvious residual antimicrobial effect during the shelf life of pork carcasses, when compared with control samples ($p<0.05$).

***Keywords**: residual antimicrobial effect, organic acids, spoilage psychrotrophs, pork carcasses*

Introduction

Several studies have been conducted to reduce the level of microbial load from the surface and depth of the carcasses (Stradford *et al*., 1999; Alakomi et al., 2000; Castelo et al., 2001; Staruch et al., 2001; Strivarius *et al.,* 2002 a, b; Ockerman *et al*., 2001a, b; Pipek *et al*., 2004, 2006; Bosilevac *et al*., 2006). These studies were carried out in the context of extending the shelf life of meat, especially when packed in vacuum, when the level of microbial load should be extremely low. Different methods have been suggested for the decontamination of carcass surfaces (steam, chlorination, trisodium phosphate, pulsatile light exposure, pulsed electric fields or ionizing radiation, organic acid solutions) (Pipek *et al.,* 1997; Stradford *et al.,* 1999; Castelo *et al.,* 2000; Strivarius *et al.,* 2002 a, b). From the organic acid solutions, lactic acid, acetic and citric acid were mostly used in different concentrations (1-5%), principally based on reducing the intracellular pH of microorganisms, thus causing their death. Organic acids have the ability to inhibit the growth of some of the alteration germs, mainly Gram negative species of the Enterobacteriaceae family, Pseudomonas genus, etc. (Van Netten et al., 1998; Podolak et al., 1996; Smulders et al., 1998, Killinger *et al*., 2010). The use of these decontamination methods, well studied, authorized and widely used in comercial slaughterhouse in some countries (USA, Australia), often raises discussions about their effectiveness, the color changes induced at the surface of the carcasses and, last but not least, the economic aspects of scale utilization at industrial slaughterhouses for meat-producing animals. Since the reduction of the carcass microbial load of the meat-producing animal carcasses represented and still represents one of the most important foodsafety objectives of the food bussiness operator, our research has aimed to assess the residual antimicrobial effect of 3%

lactic and acetic acid solutions regarding the load and configuration of psychrotrophs at porcine carcasses.

Material and methods

During October 2016 and December 2016, 18 pork meat samples were collected from a comercial slaughterhouse in Transylvania. Collected samples were sprayed with 3% acetic acid and lactic acid solutions by spraying on the surface of meat samples (2.5-3 ml/100 cm^2). Each sample was divided into three sub-samples, from which two were treated with 3% organic acids solutions and one was the control sample (not decontaminated). Experimental design were carried out over a 14-day period (shelf life of the pig carcasses), with microbial analyses at day 0, 1, 5, 9, 14. After spraying with organic acid solutions, the samples were kept at 2-4°C for 24 hours, and the following microbiological determinations were carried out: total load of psychrotrophic germs and isolation of microorganisms from the genera *Pseudomonas, Aeromonas, Yersinia* and *Enterobacteriaceae* family. Psychrotroph plate count was performed according with the protocol described by Nottingham *at. al* (1982). For isolation of psychrotrophs specific selectiv media were used, as follows: Aeromonas and Pseudomonas - GSP agar (Merck), Yesinia - CIN agar (Merck), Enterobacteriaceae – VRBD agar (Merck). Serial decimal dilutions (10^{-1} : 10^{-6}) were obtained from 10 grams of meat and 90 ml water buffered peptone. Spreading method was used to inoculate 0.1ml of inoculum on to the surface of 2 Petri plates. Incubation was realized at 20°C, for 72 hours. Biochemical confirmation test was realized using API 20 E and API 20 NE (Biomerieux). Statistical analysis was realized using Origin 8.5 software by comparison of means by analysis of variance through ANOVA test. The statistical interpretation of the results was realized according to the probability indicator: $p \leq 0.05$ (confidence level 95%). Result were depicted as log CFU/cm^2.

Results and discussions

The initial psychrotrophs load of the control sample was 3.83 ± 0.38 log CFU/cm^2, decrease after 24 hours to 3.53 ± 0.21 log CFU/cm^2, Afterwards showing an ascending trend throughout the experiment, reaching 5.67 ± 0.38 log CFU/cm^2 on day 14^{th}, maximum limit being exceeded on the 9^{th} day of the experiment. 3% acetic acid solution produced a markedly reduction of microbial load after 1 day, to 2.87 ± 0.31 log CFU/cm^2. Then the psychrotrophs presented an increasing trend in the following days, reaching 5.04 ± 0.26 log CFU/cm^2, at the end of the experiment. Similar with the effect of acetic acid, lactic acid reduced microbial load after 24 h to 2.29 ± 0.14 log CFU/cm^2, Afterwards an increasing trend was observed until the end of the experiment when the value of ± 0.23 log CFU/cm^2 (Figure 1).

Enterobacteriaceae load showed a constant increase during the experiment, starting from an initial count of 2.31 ± 0.14 log CFU/cm^2 to 4.54 ± 0.2 log CFU/cm^2. Following spraying with 3% acetic acid solution, *Enterobacteriaceae* load decreased to 0.51 ± 0.21 log CFU/cm^2, then the evolution was ascendant by the end of the experiment, when reach 2.75 ± 0.26 log CFU/cm^2, the maximum admitted limit being exceeded on day 7. A similar evolution was noticed in the sample treated with the 3% lactic acid solution, but the inhibitory effect was less intense compared to acetic acid, but no significant differences between organic acid solution were identified ($p>0.05$) (Figure 2).

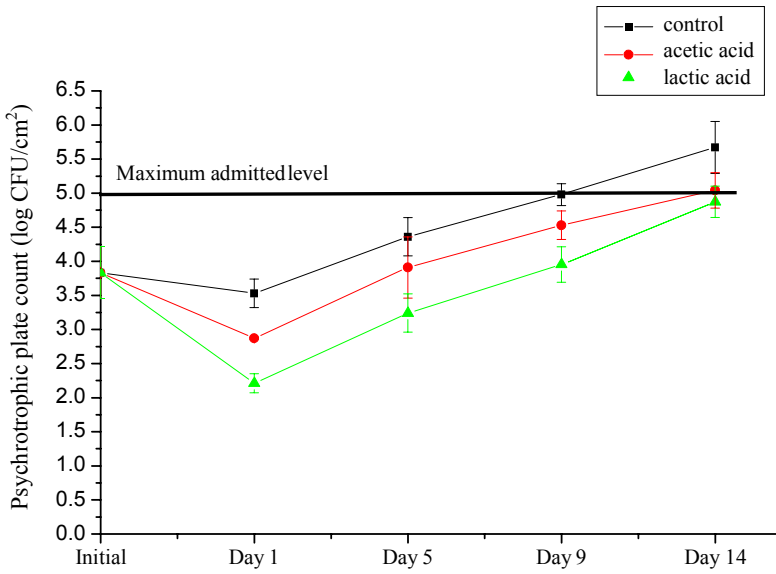

Figure 1. Decontamination effect of 3% organic acid regarding psychrotrophic plate count at the surface of pork meat (n=6)

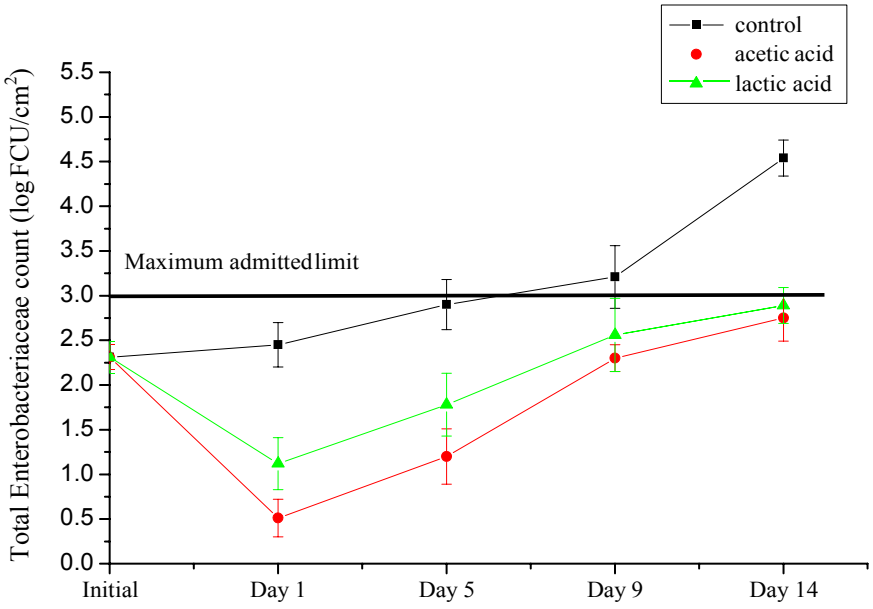

Figure 2. Decontamination effect of 3% organic acid regarding *Enterobacteriaceae load* at the surface of pork meat (n=6)

Following spraying of 3% lactic acid solution, initial microbial load of *Aeromonas* spp. decreased slightly from 2.40 ± 0.26 log CFU/cm^2, to 0.95 ± 0.14 log CFU/cm^2 after 24 h, to 0.76 log CFU/cm^2 in the 5th day of the experiment. Starting with the 9th day, *Aeromonas* spp. was totally inhibited. not on the last day of the experiment. Acetic acid solution produced total inhibition of *Aeromonas* spp. after 24 hours since spraying (Figure 3). Based on these results we can mention that aeromonads are very sensitive to the action of organic acids and especially to 3% acetic acid solution. Significant differences were recorded when compared acid with lactic acid ($p<0.05$).

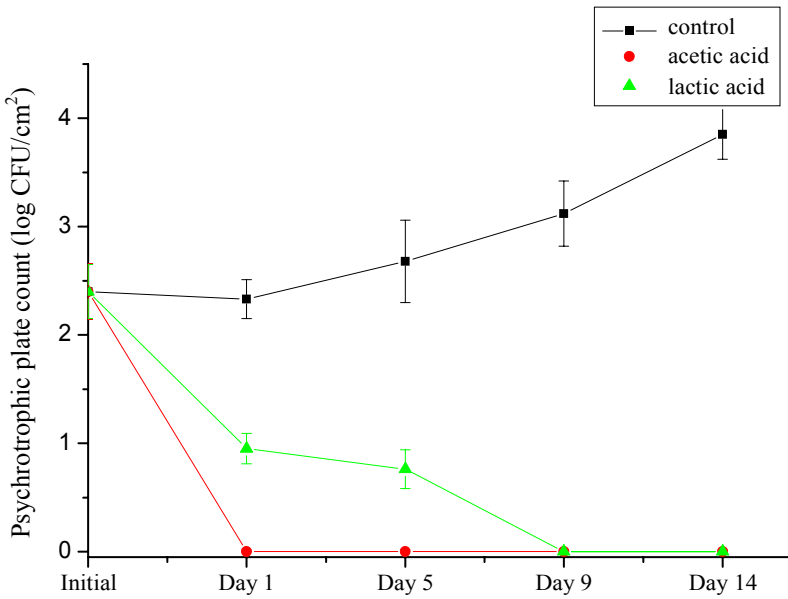

Figure 3. Decontamination effect of 3% organic acid regarding *Aeromonas* spp. at the surface of pork meat (n=6)

In the case of the control sample, *Pseudomonas* spp. load showed a moderate increase until day 5, when they reached the value of 4.06 ± 0.35 log CFU/cm^2, then the pseudomonads showed a more pronounced ascendant evolution, reaching at the end of the experiment 5.21 ± 0.39 log CFU/cm^2, the recommended maximum limit (5.0 log CFU/cm^2) being exceeded on day 13.

Both solutions of organic acids used caused a decrease of pseudomonads load after 24 hours (1.5 log CFU/cm^2 in case of 3% acetic acid). Afterwards, the results showed a more obvious effect regarding reduction of *Pseudomonas* spp. in case of acetic acid, but no differences were noticed between organic acid solution ($p>0.05$). Then, the evolution in dynamics was ascending until the last day of the experiment, when the pseudomonads reached 4.33 ± 0.32 log CFU/cm^2 in case of acetic acid, respectively 5.67 ± 0.40 CFU/cm^2 in case of lactic acid (Figure 4). Significant differences were recorded in case of control samples in comparison with those treated with lactic and acetic acid $p<0.05$).

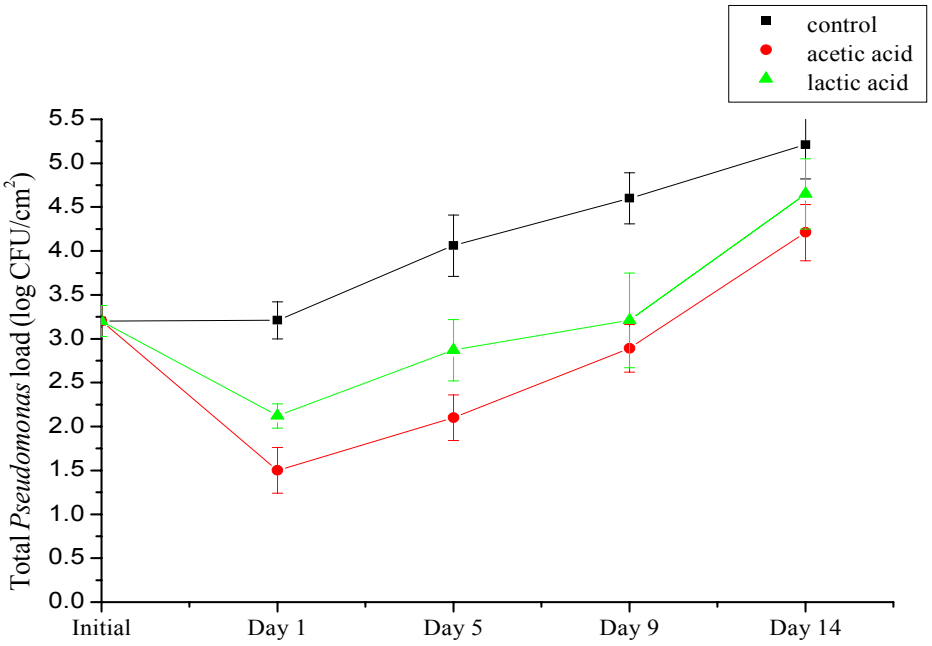

Figure 4. Decontamination effect of 3% organic acid regarding *Pseudomonas* spp. at the surface of pork meat (n=6)

In case of control sample, *Yersinia* spp. presented a ascendant evolution during the experiment, ranging from an initial load of 2.86 ± 0.28 log CFU/cm^2 to 4.78 ± 0.29 log CFU/cm^2 in the 14th day of the experiment. Acetic acid solution applied at the surface of pork meat samples produced after 24 hours a complete inhibition of *Yersinia* spp., while lactic acid solution produced a moderate reduction until day 9 when they did not were identified (Figure 5). Significant differences were recorded in case of control samples in comparison with those treated with lactic and acetic acid $p<0.05$). Similar results, reported by Mead *et al.* (1995), regarding preventive control methods for red meat, has shown that the use of 2.4% lactic acid solution for the decontamination of porcine carcasses has reduced the load with Enterobacteriaceae by 0.9 log CFU/cm^2 and the total number of germs with 1.0 log CFU/cm^2. Also, Pipek *et al*. (2006), highlighted the residual antimicrobial effect of the 3% lactic acid solution on the total germ load for a period of 5 days.

Castelo *et al.* (2001), in a dynamic study on various methods of inhibiting the psychotropic germs from the surface of pig carcasses, revealed that, following treatments with 2-3% lactic acid solutions, the total load remained constant up to 7 days at values of 3.52 log CFU/cm^2, the most effective inhibition method, which did not produce sensory changes in the carcasses, was the use of lactic acid after the final cleaning of the carcasses.

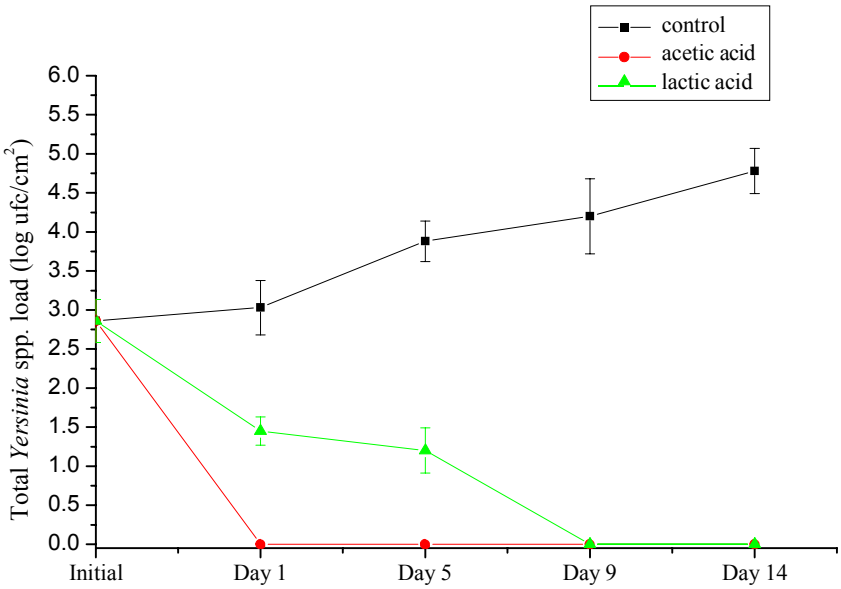

Figure 5. Decontamination effect of 3% organic acid regarding *Yersinia* spp. at the surface of pork meat (n=6)

Conclusions

The most sensitive psychrophic bacteria regarding the decontamination effect of lactic and acetic acid were *Aeromonas* spp. and *Yersinia* spp., both species being completely inhibited after 24 hours since application. For all microbiological criteria analyzed, lactic and acetic acid shown an obvious residual antimicrobial effect during the shelf life of pork carcasses, when compared with control ($p<0.05$). These methods of decontamination should not be used as basic methods of reducing initial microbial load of germs. It should also, not be considered as a substitute for the use of appropriate methods of preventing microbial contamination at the slaughter or processing units of meat, because most of these methods have only a limited effect of reducing germs or pathogens, which depends directly on the initial level of contamination.

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